Nidacon

Product Manual

Nidation

1. The building of a nidus, a nest, as with birds.
2. Implantation of the fertilized ovum (zygote) and the building of a nest in the endometrium, the placenta.

Conception

1. The union of male and female gametes, the sperm and egg.
2. An impression or idea

Contents

Nidacon

Introduction

Nidacon International AB manufactures and sells Medical Devices mainly for Assisted Reproduction Technologies (ART), with IVF, ICSI and insemination (IUI) solutions. The company was founded in 1996 by Assoc. Prof. Paul V. Holmes MSc, PhD, DrMedSc, an embryologist and endocrinologist from the Dept. of Obstetrics and Gynaecology at Sahlgrenska University Hospital in Gothenburg, Sweden.

Nidacon considers many different factors when designing its products. We hope that the attention to detail has helped to create products which will lead to better results. We aim to work in close relation with our customers; they are the cornerstones of our research department.

We take pride in the development of our products and make sure we respond to the needs of our customers and research colleagues. All our products are developed in close cooperation with professionals in the different fields.

One of the first products to result from the company's research and development, PureSperm®, was introduced onto the market in November 1996. It has gained rapid acceptance and is now the global market leader for isolation and preparation of sperm used in human assisted reproduction. It was the first product of its kind to achieve both 510(k) clearance from the US FDA and CE marking with the European authorities.

Quality

Nidacon is certified according to SS-EN ISO 9001 (implemented 2000-12-15) and SS-EN ISO 13485 (implemented 2003-08-15). The management system secures continued development of the organisation.

We register our products according to the valid directives and requirements for all different countries. This also ensures our high quality on the market and it shall continue to be our beacon.

Nidacon intends to always maintain the high quality of its products and, in order to achieve this, all batches are tested at Nidacon before they are cleared for the market. Sterility controls are performed on each batch manufactured, the endotoxin level is measured and biological efficacy tests are carried out. A batch is only released for sale if it meets specific criteria.

Each batch is accompanied by a quality assurance certificate which records the results of the tests. Using this rigorous quality control system, we ensure that each batch meets the correct standards. Consequently the customers are secure in the knowledge that our products are reliable and will provide good results when used correctly.

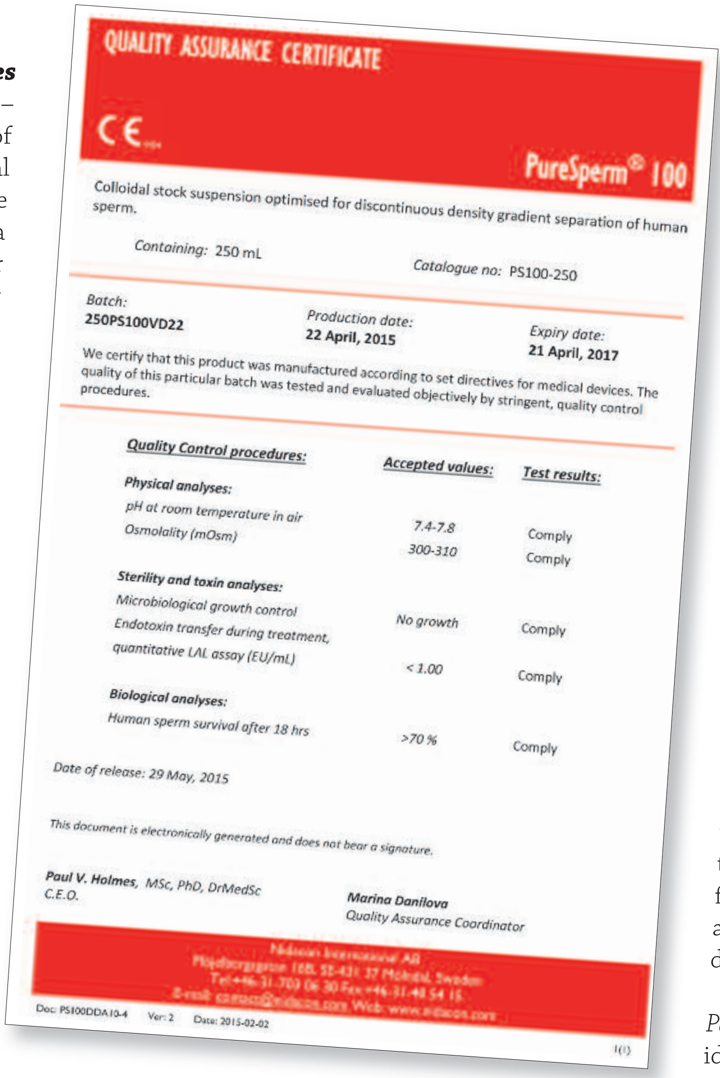
Quality Assurance tests

Physical analyses
pH – tested on every batch during production and after bottled product at room temperature in air.

Osmolality – tested on every batch during production and after.

Sterility and toxin analyses
Microbiological growth control – performed after production of a batch and involve bacterial and fungal growth assays. The assays are performed under a period of 2-3 weeks in order to be able to detect any growth. They are done by the Bacteriological Laboratory of Sahlgrenska University Hospital, Gothenburg, Sweden, an accredited, independent state laboratory.

Endotoxin detection – This assay is done with an FDA-approved, Limulus Amoebocyte Lysate (LAL) test using a quantitative spectrophotometric method in order to obtain real values with the units EU/mL, according to the U.S. Pharmacopoeia. The test is done by the accredited laboratory of the Microbiology Institute at the Sahlgrenska University Hospital of Gothenburg, Sweden.



QUALITY ASSURANCE CERTIFICATE

CE

PureSperm® 100

Colloidal stock suspension optimised for discontinuous density gradient separation of human sperm.

Containing: 250 mL *Catalogue no:* PS100-250

Batch: **250PS100VD22** *Production date:* **22 April, 2015** *Expiry date:* **21 April, 2017**

We certify that this product was manufactured according to set directives for medical devices. The quality of this particular batch was tested and evaluated objectively by stringent, quality control procedures.

Quality Control procedures:	Accepted values:	Test results:
Physical analyses:		
pH at room temperature in air	*7.4-7.8*	Comply
Osmolality (mOsm)	*300-310*	Comply
Sterility and toxin analyses:		
Microbiological growth control	*No growth*	Comply
Endotoxin transfer during treatment, quantitative LAL assay (EU/mL)	*< 1.00*	Comply
Biological analyses:		
Human sperm survival after 18 hrs	*>70 %*	Comply

Date of release: 29 May, 2015

This document is electronically generated and does not bear a signature.

Paul V. Holmes, *MSc, PhD, DrMedSc*
C.E.O.

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Doc: PS100DDA10-4 Ver: 2 Date: 2015-02-02

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Biological analyses
Human sperm test – The biological efficacy assay involves assessment of, yield motility, viability measured both subjectively and using computer assisted sperm analysis (Hamilton-Thorne, IVOS). Each batch is tested biologically using human semen samples. The samples are separated into two parts, the one part being used for control and the second part being used for preparing sperm with the new batch. The test-batch results are compared with the results from the control.The analyses provide a count of the sperm per mL, the sperm activity is graded and the activity is also expressed as a percentage of the total sperm. All data are recorded from before and after the separation and purification, and are compared to the control, i.e. using an earlier, already approved batch.

Human Sperm Survival test for oil – Prepared sperm are covered by oil and incubated overnight in 37°C, 5-6% CO_2. Percentage of motile sperm on day 2.

Mouse Embryo Assay (MEA) for bottles etc. – is used to assess the in vitro growth and development of pre-implantation embryos exposed to the test item. The assay predicts embryo toxicities in medical devices or related products to be used for assisted reproductive technology (ART).

Mouse Embryo IVF Assay for media – a sensitive assay mimicking the human IVF procedure. The preferred assay to screen assisted reproductive technology supplies for toxicities impairing male and female gamete fecundability and subsequent embryo development capacity.

Peroxide analyses – the peroxide level is measured using a QuantiChrom™ Peroxide Assay .The improved method utilises the chromogenic Fe3+-xylenol orange reaction , in which a purple complex is formed when Fe2+ provided in the reagent is oxidised to Fe3+ by peroxidise present in the sample.

Functional analysis/ Efficacy test – used to prove the efficacy and function of the products.

Visual control – constant visual control during production, filling, labeling and final control of chosen ready packages.

Shelf life

Nidacon is conscious of customer requirements and always tries to provide products which are convenient. This convenience includes ease of transportation and long shelf life. Therefore, most of the products have a shelf life of one to two years at room temperature.

All ingredients are chosen for their temperature tolerance and their stability in aqueous solution. Rigorous shelf life testing has been carried out in Nidacon's laboratory to ensure that the theoretical stability of the salt formulations is matched by their actual stability when combined in the product.

Packaging

The packaging for Nidacon's products has received the same care and attention to detail as the design of the products.

Bottles – For most of our products we have chosen borosilicate glass instead of sodium silicate glass to avoid the leaching of sodium from the bottles into the contents during the long shelf life. Research in our laboratory has shown that sufficient sodium ions can leach from a sodium silicate bottle to have a negative effect on the development of two-cell mouse embryos. Therefore, we avoid exposing all cells to raised sodium-ion levels in the products by packaging in borosilicate glass.

Stoppers – Based on embryo-toxicity testing of three types of commercially available rubber stoppers approved for pharmaceutical use today, Nidacon chose silicone rubber as the material for the stoppers. We found that both natural latex rubber and butyl rubber are toxic to embryos, preventing development and possibly causing embryonic death. Silicone rubber did not have any detrimental effect, allowing embryonic development and hatching to proceed as usual. Therefore, stoppers made from pharmaceutical silicone rubber were chosen for our products.

This convenience includes ease of transportation and long shelf life.

Background

Under normal physiological circumstances, sperm undergo a series of maturation changes after ejaculation which enables them to negotiate the different sections of the female reproductive tract, and eventually locate and fertilise the egg. If sperm are to be used for ART, it is essential that any product which is used for sperm preparation must match the sperm's physiological requirements as closely as possible. If sperm are stimulated excessively, particularly ionically, they become "hyperactive", a process which results in the sperm using up its energy resources and dying before fertilisation is achieved.

Therefore, the pH and osmolality of the sperm solutions must be adjusted very specifically to avoid ionic shock and subsequent hyperactivation.

Product composition

The component salts of Nidacon's products are balanced with specific regard to the ion composition of both the ejaculate and the female reproductive tract. This balance ensures a smooth transition from ejaculate to fertilisation medium via the gradient and wash.

Buffer

The zwitterion buffer, HEPES, is included to maintain the pH of the PureSperm® gradient and PureSperm® Wash while working with the sperm on the bench. Fluids designed to maintain pH in a CO_2 environment, i.e. in the incubator, are unsuitable for use outside the incubator as they do not possess sufficient buffering capacity to maintain the pH.

Fluctuations in pH and temperature are detrimental to sperm survival on the bench. In addition, HEPES has an anti-oxidant effect, reducing reactive oxygen species (ROS) which can be damaging in the sperm preparation.

Glucose

Glucose is a component of PureSperm® products. Glucose is the primary energy substrate available to sperm in the human female reproductive tract.

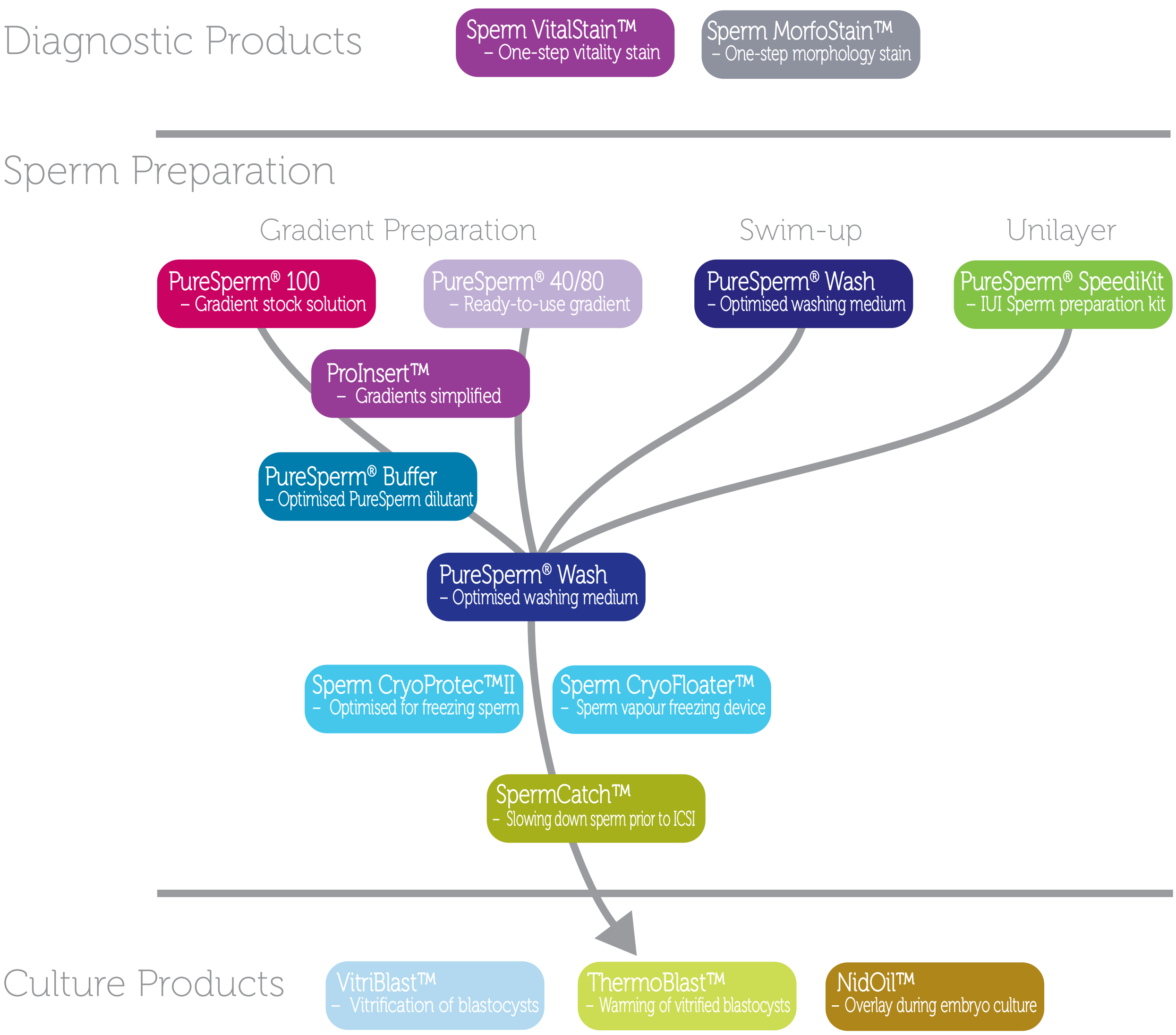
Antibiotics

Antibiotics are not included in our products for several reasons. Penicillin G, a commonly used antibiotic in cell culture medium, only lasts for approximately 10 days in aqueous solution, being inactivated after this time and the degradation products are cell-toxic. Furthermore, this antibiotic is ineffective against some of the bacteria most commonly found in semen. Streptomycin and gentamycin are cytotoxic. Gentamicin, in particular, has been shown to be toxic to embryos.

Therefore, it seems prudent to avoid including potentially spermicidal components in sperm preparation fluids. Moreover, bacterial contamination in the ejaculate is removed by density gradient preparation. Therefore, the absence of antibiotics in the gradient will not be detrimental to the sperm preparation, and avoids exposing the sperm to potentially toxic compounds.

Additives and Phenol Red

No preservatives or unstable ingredients are added to Nidacon products. In addition, we have decided not to use phenol red in our media, since it has been proven to have estrogenic effects. Gametes have receptors for estrogen and they can be affected by its presence. For instance, it has been shown that estrogen inhibits sperm motility and the acrosome reaction.



Ordering information

Cat. No.	Description	Size
PSK-020	***PureSperm® 40/80***	2 × 20 mL
PS40-100	***PureSperm® 40***	100 mL
PS80-100	***PureSperm® 80***	100 mL
PS100-100	***PureSperm® 100***	100 mL
PS100-250	***PureSperm® 100***	250 mL
PS100-1000K	***PureSperm® 100***	4 x 250 mL
PSB-100	***PureSperm® Buffer***	100 mL
PSW-100	***PureSperm® Wash***	100 mL
PSW-020	***PureSperm® Wash***	2 × 20 mL
PSSK-070	***PureSperm® SpeediKit***	10 patient preps
SC-100	***SpermCatch™***	6 × 100 µL

Cat. No.	Description	Size
SCP-020	***Sperm CryoProtec™II***	2 × 20 mL
NO-100	***NidOil™***	100 mL
NO-300	***NidOil™***	300 mL
SVS-010	***Sperm VitalStain™***	2 × 10 mL
SMS-250	***Sperm MorfoStain™***	250 mL
VBK-010	***VitriBlast™Kit***	3 × 10 mL
TBK-010	***ThermoBlast™Kit***	4 × 10 mL
PI15-5	***ProInsert™***	5 kits
CFV-001	***Cryofloater vial***	1
CFV-002	***Cryofloater straw***	1

10 items or more on one purchase order gives 5% discount.
We have distributors in most countries, for a complete list take a look at our web page www.nidacon.com

PureSperm® 100

Sterile, silane-coated, silica colloid in an isotonic salt solution. It is optimised for the preparation of discontinuous density gradients for the separation and purification of human sperm.

Shelf life 2 years. CE 0434

QA *Sterility – Osmolality – Endotoxin*
pH – Human Sperm Survival

Components	
Silane-Coated Silica	*Purified water*
NaCl	*CaCl*
Glucose	*KCl*
EDTA	
HEPES	

PureSperm® 40 PureSperm® 80

Ready-to-use density gradient solutions, 40 and 80%. They make work in the lab easier and minimizes the risk for mistakes.

Shelf life 2 years. CE 0434

QA *Sterility – Osmolality – Endotoxin*
pH – Human Sperm Survival

Components	
Silane-Coated Silica	*KCl*
NaCl	*Citrate*
Glucose	*Lactate*
Pyruvate	*HEPES*
EDTA	*Purified water*

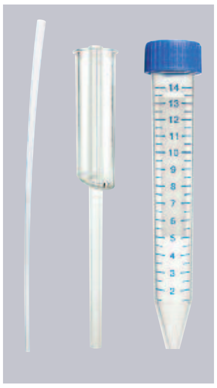
PureSperm® Buffer

Balanced salt solution designed specifically for diluting PureSperm® 100 to make up the layers of different densities for a discontinuous gradient. The optimised formulation of PureSperm® Buffer is designed to maximise sperm survival during gradient centrifugation.

Shelf life 2 years. CE 0434

QA *Sterility – Osmolality – Endotoxin*
pH – Human Sperm Survival

Components	
NaCl	*EDTA*
KCl	*Citrate*
HEPES	*Glucose*
Lactate	*Purified water*
Pyruvate	



ProInsert™

The ProInsert™ reduces the risk of recontamination of the sperm pellet during sperm retrieval after a density gradient separation. The ProInsert™ is a safe and easy-to-use device, designed for use with Nidacon products.

Shelf life 2 years.

QA *Efficacy test – MEA – Sterility – Human Sperm Survival*

Components
Centrifuge tube containing
the ProInsert™
PRP (Pellet Retrieval Pipette)

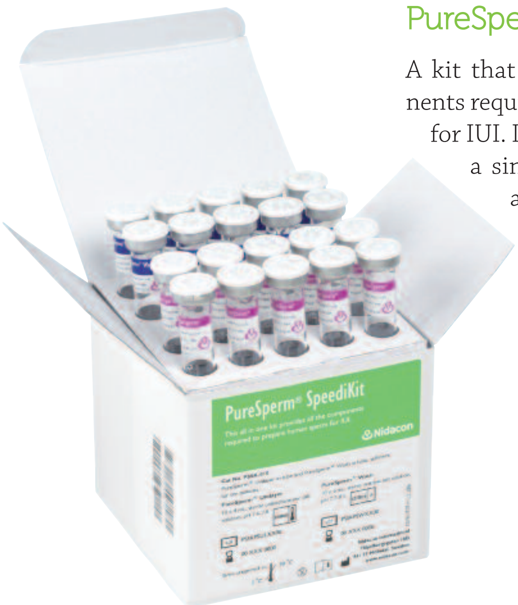
PureSperm® Wash

Sterile isotonic salt solution. Optimized for washing the sperm recovered from density gradient preparations, for use in swim-up procedures, for extension of sperm prior to IUI or as a medium for maintaining sperm.

Shelf life 1 year.

QA *Sterility – Osmolality – Endotoxin pH – Human Sperm Survival*

Components	
NaCl	*Pyruvate*
$MgSO_4$	*KCl*
EDTA	*KH_2PO_4*
Purified water	*Glucose*
HEPES	*$NaHCO_3$*
Lactate	
hSA (human serum albumin)	



PureSperm® SpeediKit

A kit that provides you with the components required to prepare 10 sperm samples for IUI. It contains ready-to-use tubes for a single layer colloid centrifugation and ready-to-use tubes with PureSperm® Wash for the washing of the pellet after centrifugation. A perfect product for the small clinic, 10 patients/kit.

Shelf life 1 year.

QA *Sterility – Osmolality – Endotoxin pH – HumanSperm Survival*

Components	
Silane-Coated Silica	*KCl*
NaCl	*Citrate*
Glucose	*Lactate*
Pyruvate	*HEPES*
EDTA	*Purified water*
$MgSO_4$	*$NaHCO_3$*
hSA (human serum albumin)	

Sperm CryoProtec™II

Sterile salt solution containing glycerol, optimised for freezing both gradient-prepared sperm and for unprocessed ejaculates. Nidacon recommends the nitrogen-vapour freezing technique, since this technique seems to provide the best results after thawing.

Shelf life 1 year. CE 0434

QA *Sterility – Endotoxin – pH*
Recovery rate after freezing and thawing

Components	
NaCl	*EDTA*
KCl	*$NaHCO_3$*
HEPES	*Lactate*
Glucose	*Glycerol*
$MgSO_4$	*Pyruvate*
KH_2PO_4	*Purified water*

CryoFloater™

A floating device , used when cryo preserving semen or prepared sperm in liquid nitrogen. It provides a constant distance between the sample and the nitrogen surface, to standardize the freezing rate.

QA – *Visual inspection*

Components
Polyethylene foam

Sperm VitalStain™

One step staining technique for assessment of sperm vitality, one of the basic tools in semen analysis.

Shelf life 2 years. CE 0434

QA pH – *Functional analysis*

Components	
NaCl	*Nigrosine*
Eosin	*Formalin*
Purified water	

Sperm MorfoStain™

Classic Romanovsky stain. It is a one-step stain optimised for assessment of sperm morphology.

Shelf life 2 years. CE 0434

QA *Morphological test*

Components	
Methanol	*May Grunwald*
Eosin Y	*Giemsa*
Methylene B	*Azur B*

SpermCatch™

For slowing down sperm prior to ICSI without using polyvinylpyrrolidone (PVP). To avoid ICSI injection of PVP, it contains only natural products for increasing the viscosity.

Shelf life 1 year.

QA *Sterility – Osmolality – Endotoxin*
pH – Human Sperm Survival

Components	
NaCl	*Pyruvate*
$MgSO_4$	*Lactate*
KCl	*EDTA*
KH_2PO_4	*Purified water*
Glucose	*HEPES*
$NaHCO_3$	*Hyaluronic acid*
hSA (human serum albumin)	

NidOil™

Sterile, light paraffin oil for use as an overlay during gamete, zygote and pre-embryo culture in the incubator, or during manipulations outside the incubator. No additives, UV-protective packaging.

Shelf life 2 years. CE 0434

QA Density – *Sterility – Endotoxin – Human Sperm Survival*
Mouse Embryo assay – Peroxides analyses

Components
Light paraffin oil

VitriBlast™

Kit for vitrification of blastocysts based on well tested formulations. Numerous publications demonstrate the effectiveness regarding both survival and pregnancy rates.

Shelf life 12 months.

QA *Sterility – Osmolality – Endotoxin – pH*
Mouse Embryo assay

Components	
NaCl	*KCl*
$MgSO_4$	*KH_2PO_4*
Glucose	*$NaHCO_3$*
Pyruvate	*EDTA*
Ficoll	*Purified water*
HEPES	*Lactate*
Sucrose	*Ethyleneglycol*
DMSO	
hSA (human serum albumin)	

ThermoBlast™

Kit optimised for warming blastocysts vitrified with VitriBlast™ Kit. Ready-to-use solutions.

Shelf life 12 months.

QA *Sterility – Osmolality – Endotoxin – pH*
Mouse Embryo assay

Components	
NaCl	*KCl*
$MgSO_4$	*KH_2PO_4*
Glucose	*$NaHCO_3$*
Pyruvate	*EDTA*
Purified water	*Sucrose*
HEPES	*Lactate*
hSA (human serum albumin)	

Background

A normal semen sample (ejaculate) is made up of seminal fluid which contains a number of different cells, cell debris, microbiological and biological substances.

The different cell types contained in semen are normal motile sperm, juvenile sperm and senescent sperm (no fertilisation function) and sperm with DNA breaks. Epithelial cells from the male reproductive tract, male immune cells and cell debris (detritus) are also present in the semen, as are bacteria and possibly viruses.

Moreover, the seminal fluid contains biologicals such as sperm decapacitating factors and reactive oxygen species (ROS), both of which negatively affect fertilisation.

After ejaculation in vivo, normal sperm quickly migrate from the liquefied semen into the uterine cervix of the female, thereby separating themselves from adverse affects of the factors previously mentioned.

In the andrology laboratory of an IVF-clinic, separation of the normal motile sperm from seminal fluid and its contents can be achieved by using either a “discontinuous density gradient” or a “swim-up”.

Positive features of a discontinuous density gradient according to Nidacon.

Feature	Density Gradients	Swim-up
Separates motile sperm from other cell types	4	4
Separates out immature, aged and dying sperm	4	—
Separates out morphologically abnormal sperm	4	—
Separates out sperm with damaged chromatin	4	—
Removes bacteria and viruses	4	—

If the density gradient has been prepared correctly, the sperm pellet should contain only functional, fertile sperm.

The use of two density-gradient centrifugation techniques and the swim-up method to seperate spermatozoa with chromatin and nuclear DNA anomalies.

D. Sakkas et al. (2000) Human Reprod.

	Initial sample	Sediment	Final prep.	Paired t-test
Swim-up	21.5±9.5	19.6±9.7	22.0±9.5	0.6
PureSperm gradient	33.9±21.2	25.0±19.8	12.4±12.6	P 0.001

The mean percentage of spermatozoa positive to Chromomycin A3 – decreased presence of protamine.

General care and use

1 All solutions should be brought up to room temperature before use to avoid the temperature fluctuations which are detrimental to sperm survival.

1 Open and reseal bottles in a laminar air-flow bench using sterile techniques to avoid contamination.

1 Store all opened bottles at 2-8°C after re-sealing.

Density Gradient Preparation

PureSperm® 100 PureSperm® 40 PureSperm® 80 PureSperm® Buffer PureSperm® Wash

Recommendations

If you have a sample with a high volume (>3mL), you can prepare two PureSperm® gradients for each semen sample. This reduces the risk of overloading a single gradient, provides security when handling tubes or recovering sperm pellets and provides two tubes to balance the centrifuge rotor.

Reagents and Equipment

PureSperm® 100 plus PureSperm® Buffer or
PureSperm® 40 and 80
Sterile Pasteur pipettes
PureSperm® Wash
Sterile 2 mL and 10 mL pipettes
Bench top centrifuge with swing out rotor

Procedure

1. If you use PureSperm® 100, dilute with PureSperm® Buffer to make your gradient solutions, for example add 2 mL PureSperm® Buffer to 8 mL PureSperm® 100 to obtain 10 mL 80% PureSperm®. Add 6 mL PureSperm® Buffer to 4 mL PureSperm® 100 to obtain 10 mL 40% PureSperm®. Instead you can use the ready-to-use PureSperm® 40 and PureSperm® 80 solutions.

2. Use a sterile pipette to add 2 mL of 80% PureSperm® to a conical tube.

3. Use a new pipette to carefully layer 2 mL 40% PureSperm® on top of the 80% layer. It is important not to disrupt the two layers and to maintain a sharp interface.

4. Layer the liquefied semen onto the gradient. We recommend that you don't take more than 1,5 mL /gradient or you risk overloading the gradient and not getting a good result.

5. Centrifuge at 300 x g for 20 minutes. Make sure that your centrifuge uses the correct g-force (use equation, p. 13). Do not use the brake.

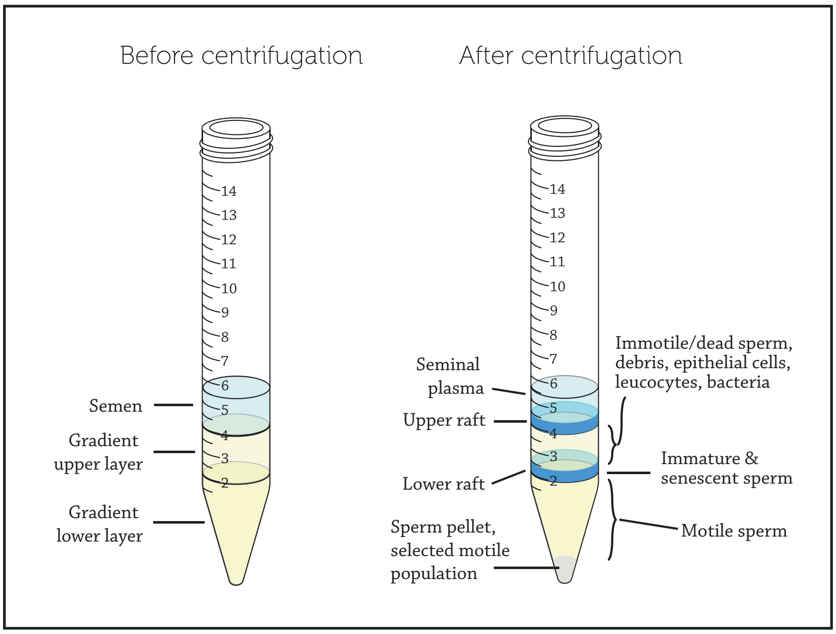
6. Aspirate in a circular movement from the surface everything except the pellet and 4-6 mm of the 80% PureSperm® layer. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5 mL.

7. Use a new pipette to aspirate the pellet (or the lowest 0.5 mL). Transfer sperm pellet to a new tube and resuspend pellet in 5 mL PureSperm® Wash. Always use a new tube with PureSperm® Wash to avoid contamination from the ejaculate. Combine sperm pellets if double procedure has been used.

8. Centrifuge at 500 x g for 10 minutes. Do not use the brake.

9. Aspirate PureSperm® Wash supernatant leaving as little liquid as possible above the pellet. If no pellet is seen, leave the bottom 0.25 mL fluid.

10. Resuspend the sperm pellet in a suitable volume of media. The sample is now ready for use.



Nidacon

Calibrate the centrifuge; to achieve the correct g force, use the equation:

$Rpm = \sqrt{[(g/(1.118 \times r))]} \times 10^3$

g = the centrifugal force

r = rotational radius, the distance (mm) from the centre of the rotor to the bottom of a centrifuge tube in the bucket when raised to horizontal position

For example; to achieve 300 x g when radius = 165 mm the centrifuge speed must be:

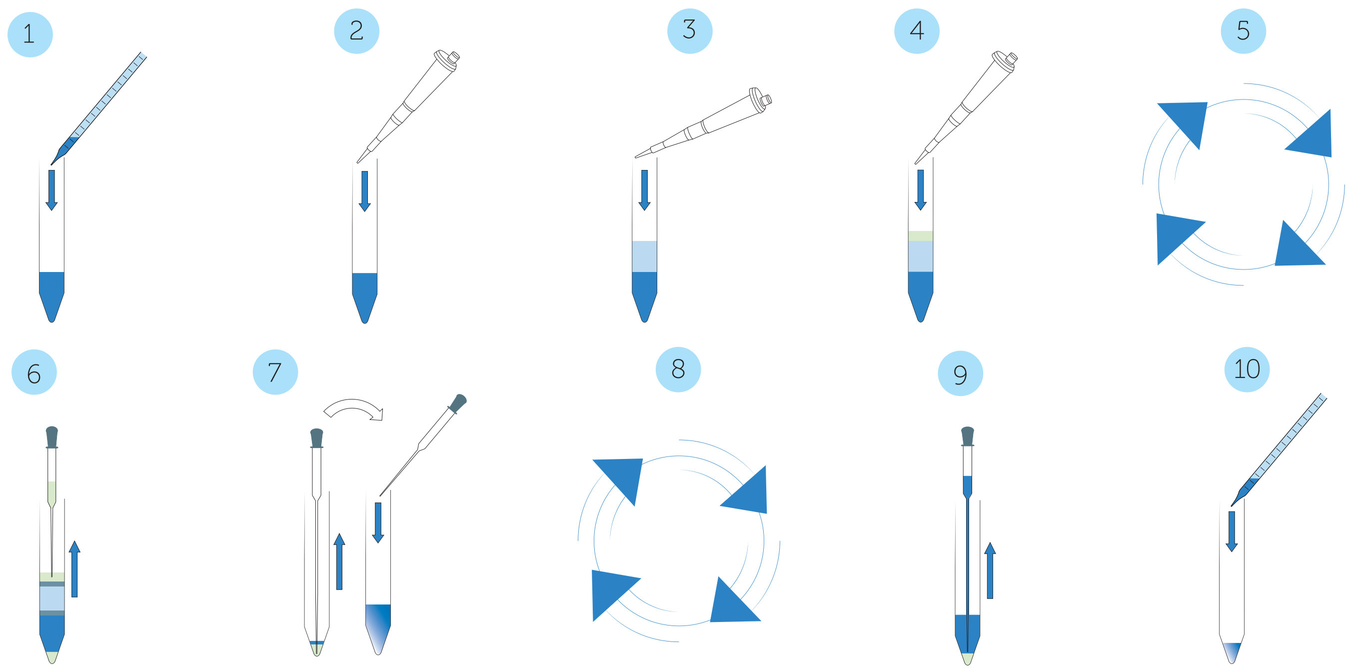
$Rpm = \sqrt{[(300/(1.118 \times 165))]} \times 10^3 = 1275$

Conversion table – concert between times gravity /x g) and centrifuge rotor speed (RPM)

http://cabinet.weblog.com.pt/arquivo/TR0040dh4-Centrifuge-speed.pdf

G Force /RPM calculator

http://drycake.com/calculator/gforce.php



Tips

- Gradients should be layered immediately prior to use but the different density solutions of PureSperm® can be prepared in advance, provided that they are stored at 4°C and brought up to room temperature before use.
- Viscous samples can be treated with PureSperm®Buffer. You simply add PureSperm®Buffer to the ejaculat, dilution 1:3, 1 part PureSperm®Buffer and 3 parts sample, incubate for 15-30 minutes at 37°C and the sample is ready for use.
- When retrieving the pellet after the gradient centrifugation, care must be taken to avoid contaminating the pellet with components of the ejaculate or upper gradient layer. Therefore we recommend that you use a new pipette after removing most of the gradient to avoid contamination, for example, by bacteria.

PureSperm® SpeediKit

Background

We especially recommend PureSperm® SpeediKit for the smaller clinics or for IUI clinics. PureSperm® SpeediKit is a rapid and efficient alternative to sperm-preps using density 'gradient' centrifugation. Everything is included in a convenient kit form for quick sperm preparation, all based on the effective centrifugation through a single layer of PureSperm® colloid, followed by rinsing the sperm with PureSperm® Wash. The kit contains both the PureSperm® colloid and the PureSperm® Wash for 10 patients, already dispensed in centrifuge tubes. You do not need an incubator.

Reagents and Equipment

Ready-to-use tubes of PureSperm® Unilayer and
PureSperm® Wash (included in the kit)

Bench top centrifuge with swing-out rotor
Sterile Pasteur pipettes

Procedure

1. Use a sterile pipette to carefully layer liquified semen (up to 1.5 mL) on top of the PureSperm® Unilayer. If you have a sample volume greater than 1.5 mL, use two tubes.

2. Centrifuge at 300 x g for 30 minutes. Do not use the brake.

3. Use a new sterile pipette to aspirate the supernatant, leaving about 5 mm of liquid above the pellet. If no pellet is seen after centrifugation, remove all fluid except the lowest 0.5 mL.

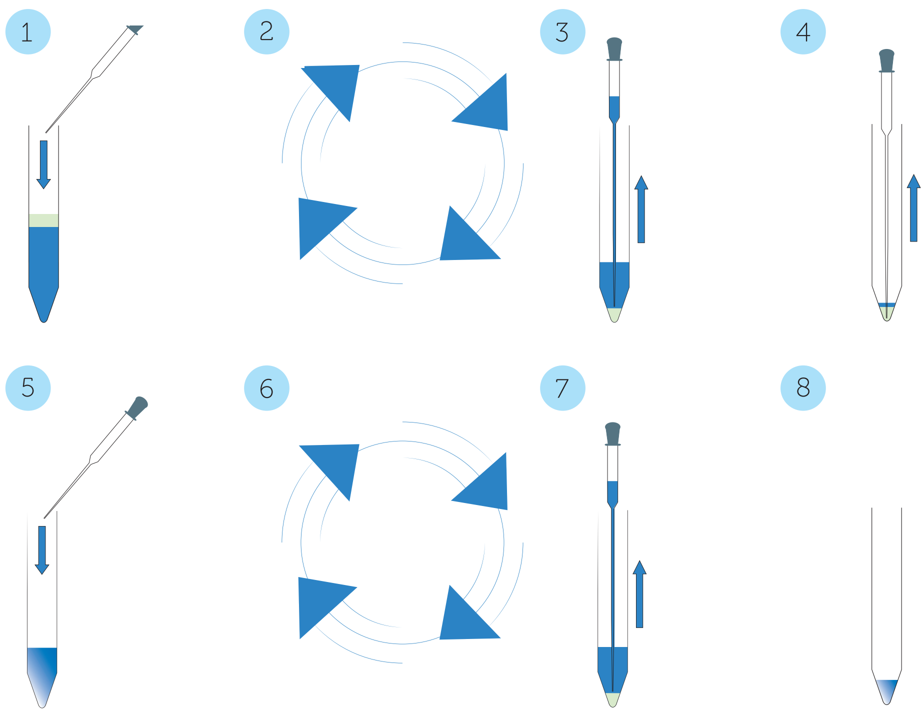
4. Use a new pipette to aspirate the pellet (or the lowest 0.5 mL).

5. Transfer sperm pellet to the tube containing PureSperm® Wash. Resuspend the sperm.

6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.

7. Use a new pipette to aspirate the supernatant. If no pellet is seen, leave the bottom 0.25 mL fluid.

8. Resuspend the pellet in the remaining PureSperm® Wash. The sperm preparation is now ready for use.



ProInsert™

Background

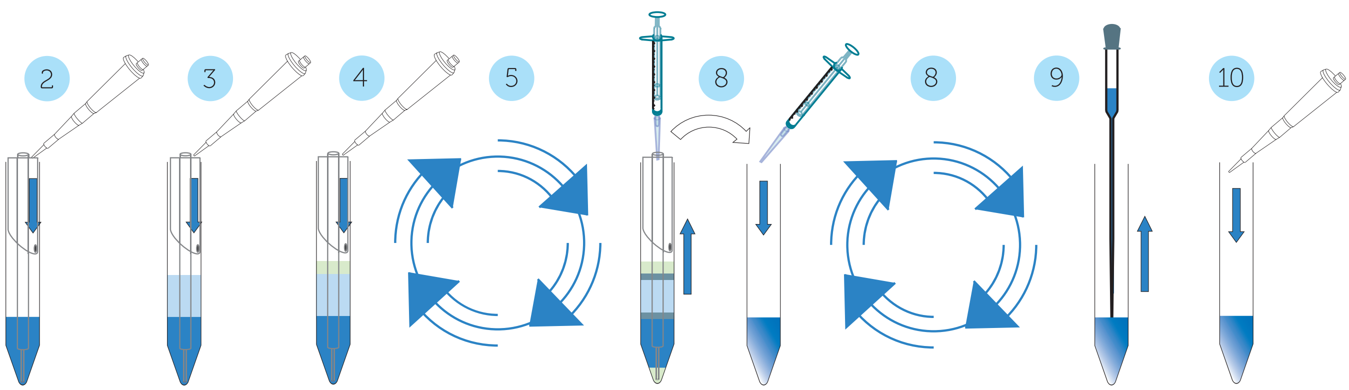
A density gradient will effectively remove lymphocytes, epithelial cells, abnormal or immature sperm, cell debris, seminal fluid, bacteria and to some extent viruses. When performing a density gradient the only means of retrieving the sperm pellet at the bottom of the tube is to either, go directly through the layers, disrupt them and risking to re-contaminate the pellet or to remove as much as possible of the layers, go through the remaining gradient and still risking to re-contaminate. Since the gradient will include many risky contaminants it is crucial that the sperm pellet is not re-contaminated. The ProInsert™ will reduce this re-contamination risk. The insert is included in the centrifuge tube from the start and after the preparation the pellet can be retrieved through a channel which leads all the way down to the pellet without coming in contact with the now contaminated gradient.

Reagents and Equipment

Centrifuge tube containing the ProInsert™
Centrifuge tube for washing media (PureSperm® Wash)
PRP (Pellet Retrieval Pipette)
Bench top centrifuge with swing-out rotor
Sterile pipettes

Procedure for using ProInsert™

1. Open a ProInsert™ Kit and remove the centrifuge tube containing the ProInsert™.
2. Use a sterile pipette to add 2 mL of PureSperm®80 to the outer channel. The gradient material will run down into the ProInsert™ chamber, out through a hole at the bottom of the chamber, and down the wall of the centrifuge tube to form a layer at the bottom of the centrifuge tube.
3. Repeat number 2 above, using a new sterile pipette tip and PureSperm® 40, again via the outer channel.
4. Use a new sterile pipette tip to carefully layer liquefied semen (up to 1.5 mL) again via the outer channel. Take care not to touch the edges of the central channel with the semen.
5. Cap the tube and centrifuge at 300 x g for 20 minutes. Do not use the centrifuge brake. Calculate the correct RPM for your centrifuge.
6. Add 5mL PureSperm® Wash to a centrifuge tube (not illustrated).
7. Attach the sperm-retrieval pipette from the ProInsert™ Kit to a 1-2 mL syringe (not illustrated).
8. Pass the pipette slowly into the ProInsert™ via the central channel, down to the sperm pellet (see graphic), be careful not to disrupt the pellet. Aspirate the sperm pellet. Retract the pipette until the tip of the pipette is safely above the liquid surface, aspirate a little air and retract the pipette from the central channel. (This procedure is to ensure that no contents in the pipette will be lost during transfer to the PureSperm® Wash). Transfer the pellet to the tube containing PureSperm® Wash. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
9. Aspirate the PureSperm® Wash supernatant, leaving as little liquid as possible above the pellet. If no pellet is seen, leave the bottom 0.25mL fluid.
10. Re-suspend the sperm pellet in a suitable volume of culture/transfer medium (e.g. PureSperm® Wash) to obtain the required sperm concentration for IVF, ICSI or IUI. The sperm sample is now ready for analysis or use.



Swim-up

PureSperm® Wash

Background

For most situations Nidacon recommends using a discontinuous density gradient for preparing human sperm from semen. However, many customers at some time need to use the swim-up technique and the most ideal product for this purpose is PureSperm® Wash.

PureSperm® Wash is a salt solution balanced and adjusted for the nutrition and long survival of human sperm. It functions exceedingly well in this role.

Recommendations

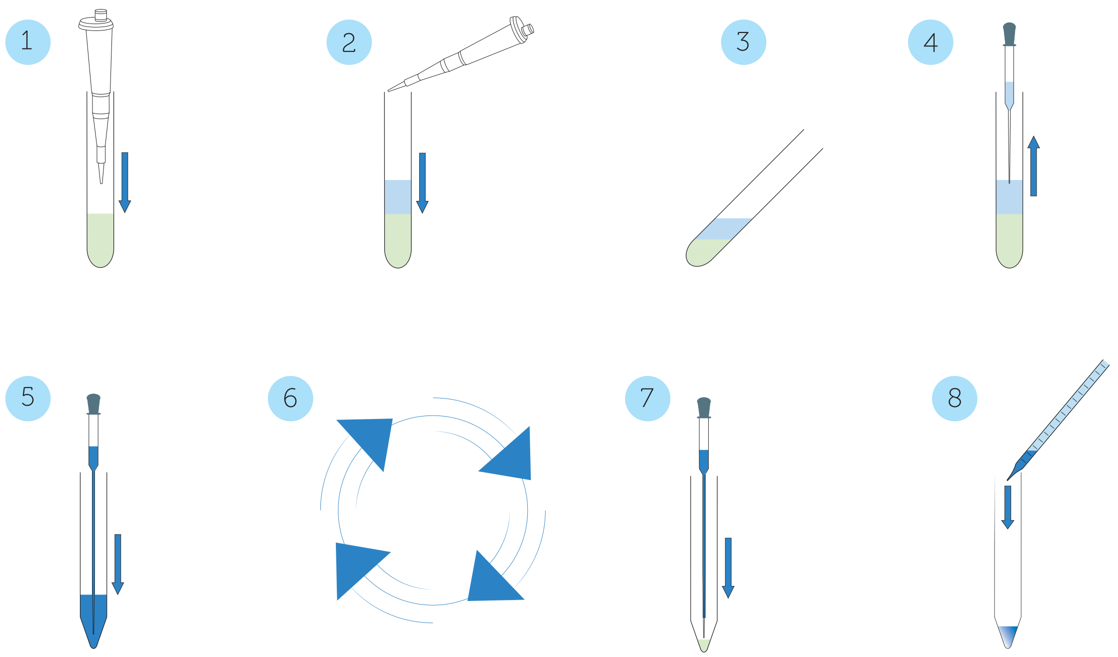
Since PureSperm® Wash does not contain any antibiotics and since swim-up cannot guarantee removal of bacterial contamination, it is recommended to add antibiotics when using swim-up to prepare sperm for ART. We recommend that you add Penicillin at a concentration of 100 U/mL.

Reagents and Equipment

PureSperm® Wash
Round bottomed centrifuge tubes
Disposable sterile conical centrifuge tubes
Sterile pipettes
CO_2 incubator
Bench top centrifuge with swing out rotor

Procedure

1. Transfer 1 mL of liquefied semen to a sterile round bottomed centrifuge tube. If the sample is too viscous, try diluting it with PureSperm® Buffer before.
2. Use a new pipette to carefully layer 1,5 mL PureSperm® Wash over the semen.
3. Without disturbing the layers, place the centrifuge tube at a 45° angle into a CO_2 incubator, at 37°C for 60 minutes.
4. Carefully remove the uppermost (0,5-1,0 mL) of medium containing motile sperm using a sterile pipette.
5. Place this fluid in a sterile conical centrifuge tube containing 5 mL PureSperm® Wash.
6. Centrifuge at 500 x g for 10 minutes. Do not use the brake.
7. Aspirate the supernatant, leaving no more than 2 mm depth of liquid above pellet.
8. Resuspend the sperm pellet in a suitable volume of medium to obtain the required sperm concentration. The sample is now ready for analysis or use.



Tips

1 If you have a viscous sample, be extra careful when you remove the upper layer after incubation. It is easy to get hold of the semen sample and disrupt the layers.

Sperm CryoProtec™II

Background

The cryoprotectant in SpermCryoProtec™II is glycerol, the proportion being reduced as far as possible to minimize toxicity to sperm, while still providing cryoprotection. Moreover, a high concentration of glucose is present as an osmotic agent to reduce intracellular water; thus diminishing damage due to ice-crystal formation.

Recommendations

Although it is possible to freeze unprocessed semen, we recommend that you prepare the ejaculate using a PureSperm® density gradient. This method removes seminal plasma as well as ROS and their sources, thereby ensuring optimal recovery of motile sperm on thawing.

Reagents and Equipment

Sperm CryoProtec™II and PureSperm® Wash
Sterile pipettes
Disposable sterile centrifuge tubes (e.g. Falcon 2075)
Disposable sterile cryopreservation vials or plastic straws
Scissors
CryoFloater™ (Nidacon)

Dilution table

Sperm Sample (µL)	SCPII™ (µL)	Sperm Sample (µL)	SCPII™ (µL)	Sperm Sample (µL)	SCPII™ (µL)
100	33	1100	367	2100	700
200	67	1200	400	2200	733
300	100	1300	433	2300	767
400	133	1400	467	2400	800
500	167	1500	500	2500	833
600	200	1600	533	2600	867
700	233	1700	567	2700	900
800	267	1800	600	2800	933
900	300	1900	633	2900	967
1000	333	2000	667	3000	1000

For other volumes than those listed; calculate:	
Volume Sperm Sample / 3 = Volume SCPII	Example: 300 µL Sperm Sample / 3 = 100 µL SCPII

Tips

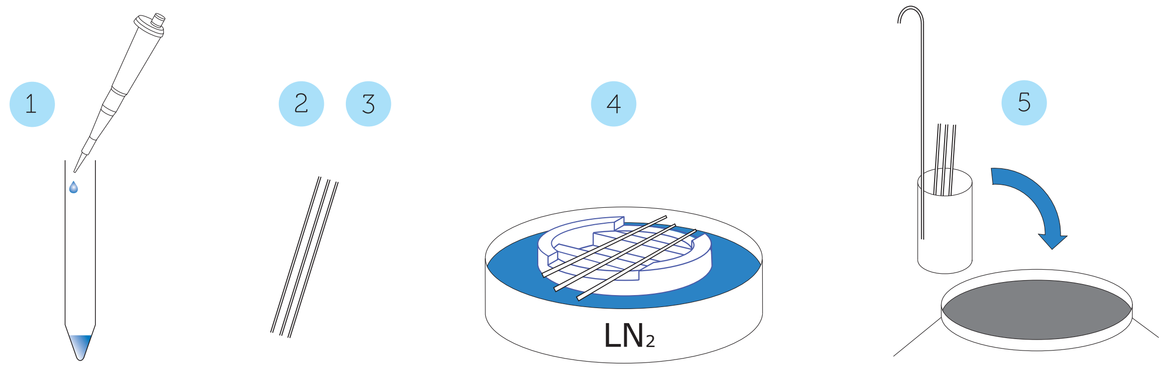
1 To avoid osmotic shock for the sperm, it is important to slowly mix Sperm CryoProtec™II with your sperm sample but don't mix for longer than 5 minutes since glycerol is toxic to cells at RT.

1 The incubation time before freezing can be reduced to 15 minutes, but we recommend 60 minutes.

Freezing of spermatozoa

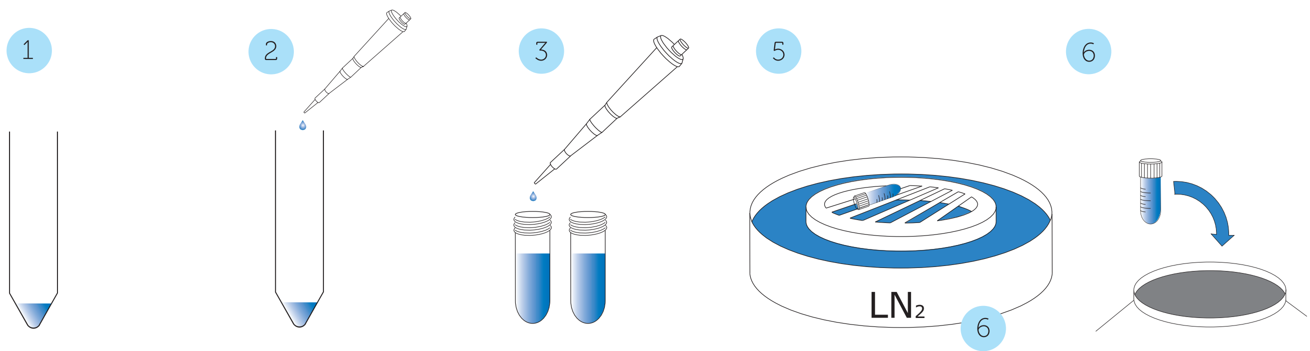
Processed ejaculate

1. Add 1 part of Sperm CryoProtec™II to 3 parts of sample (see dilution table) ensuring thorough mixing after adding each drop.
2. Fill straws with sperm suspension or aliquot into vials.
3. Equilibrate straws or vials in refrigerator for 30-60 minutes.
4. Place the straws horizontally in nitrogen vapour, above the liquid nitrogen surface on a piece of styrofoam (CryoFloater™). Leave for 30 minutes.
5. Transfer the straws quickly into the liquid nitrogen and, thereafter, store in liquid nitrogen. Do not touch the straw with your hand.



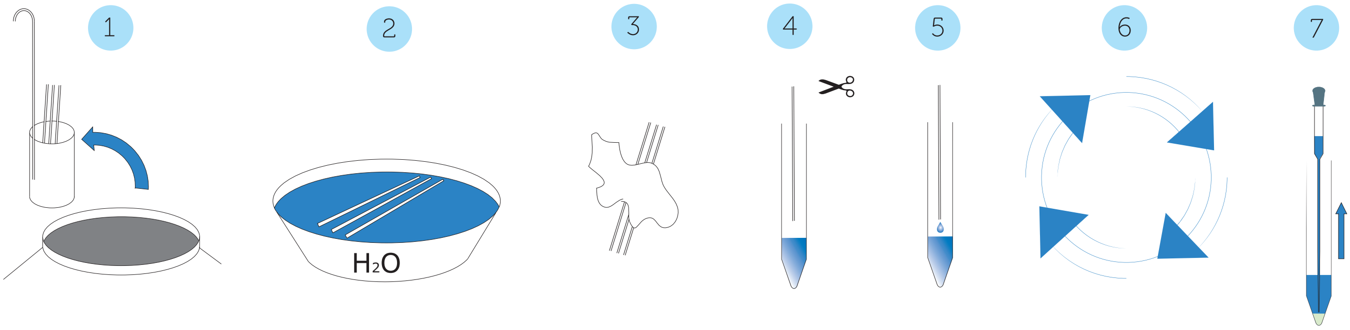
Unprocessed ejaculate

1. Measure the volume of the ejaculate.
2. Mix ejaculate with SCPII, dilution 1:3 (see table), ensuring thorough mixing after adding each drop in order to avoid osmotic shock.
3. Transfer 0.8-1.8 ml of the mixture to 2 ml cryovials.
4. Place the vials in the fridge (4-5°C) for 30 min.
5. Freeze the vials horizontally in the freezer or in nitrogen vapour, above the liquid nitrogen surface on a piece of styrofoam (CryoFloater™). Leave for 30 min.
6. Store in liquid nitrogen.



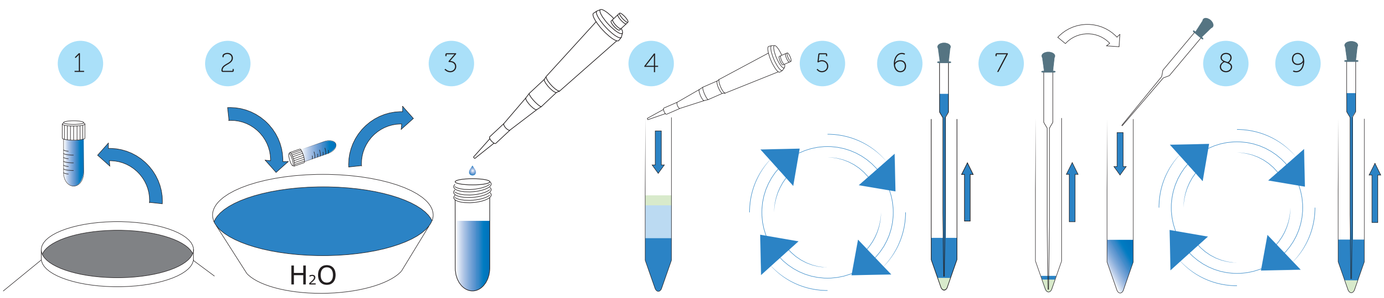
Thawing procedure processed ejaculate

1. Remove the straws from the liquid nitrogen tank.
2. Place straws in water at 37°C for 30 secs.
3. Dry surface of straw.
4. Cut one end of straw.
5. Hold the straw over a tube with 5 mL PureSperm® Wash and cut the other end of the straw. Any sperm suspension remaining in the straw can be expelled using a pipette.
6. Centrifuge at 500 x g for 10 minutes. Do not use thebrake.
7. Aspirate PureSperm® Wash supernatant leaving as much liquid as required for desired concentration. If no pellet is seen, leave the bottom 0.10 mL fluid.
8. The sample is now ready for use.



Thawing procedure unprocessed ejaculate

1. Remove the vials from the liquid nitrogen tank.
2. Place vials in water at 37°C until all ice crystals are gone, approximately 2-3 min.
3. Dilute the thawed material with 0.5 ml Pure-Sperm®Wash.
4. Prepare the thawed material on a 40% and 80% PureSperm® density gradient. Use 1 ml of each for the gradient and layer not more than 1 ml of the thawed ejaculate onto the gradient.
5. Centrifuge at 300 x g for 20 min.
6. Aspirate everything except the pellet and 4-6 mm of the PureSperm® 80% layer.
7. Use a new pipette to aspirate the pellet. Transfer to a new tube containing 4 ml PureSperm® Wash.
8. Centrifuge at 500 x g for 10 min.
9. Aspirate PureSperm® Wash supernatant and the sample is now ready for use.



SpermCatch™

Background

SpermCatch™ is an alternative to PVP (polyvinyl-pyrrolidone) which today is the most common substance used for slowing down sperm prior to ICSI. However, PVP has been reported to cause problems, such as damaging the sperm plasma membrane. It may also interfere with sperm nucleus decondensation.

SpermCatch™ is a solution without PVP, contains instead hyaluronic acid which is a natural component. Several studies have shown that SpermCatch™ gives the same or even better results than PVP. Since SpermCatch™ is a solution containing hyaluronic acid, see the following reference for the advantages. (ref 21)

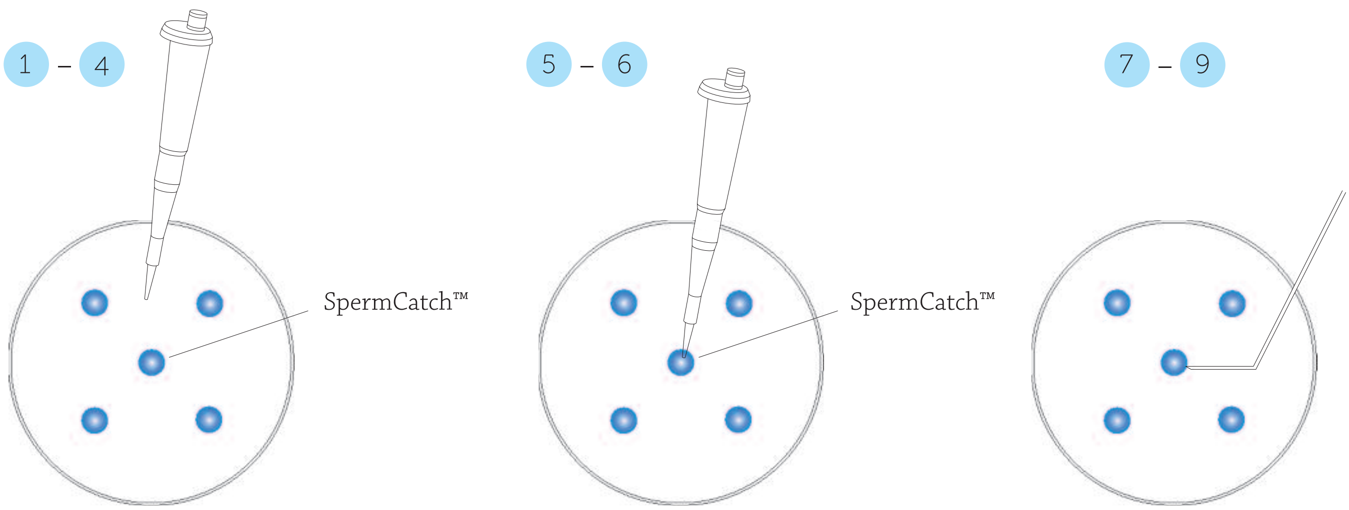
Reagents and Equipment

SpermCatch™
NidOil™
Injection media

Sterile pipettes
ICSI equipment
Petri dish

Procedure

1. Place a 10 µL drop of SpermCatch™ in the middle of a petri dish.
2. Place 4 drops of 10 µL injection media around the SpermCatch™ drop in the petri dish.
3. Immediately cover the drops with NidOil™.
4. Incubate for 30 minutes in CO_2 environment at 37°C.
5. Add 1 µL of prepared sperm suspension to the middle of the SpermCatch™ drop.
6. Incubate for 10 minutes in CO_2 environment at 37°C.
7. Fill your injection pipette with SpermCatch™ to avoid the sperm sticking to the inside of your pipette. It will also help you to make a controlled injection.
8. Immobilise the individual sperm by using the injection pipette to "knick" the sperm tail.
9. Aspirate the immobilised sperm.
10. Move to one of the oocyte droplets. Focus on the oocyte and position the oocyte with the holding pipette. Bring down your injection pipette and inject the sperm. Make sure that the oolemma is broken before you expel the sperm.



Tips

- ICSI dishes must be prepared quickly to avoid osmolarity changes in the media. Only make two at a time.
- It is convenient to have two dishes per patient.

NidOil™

Background

Mineral oil to overlay the embryo culture is used extensively in IVF laboratories. NidOil™ is a paraffin oil which has been specifically chosen and then treated in our production facilities to ensure that its purity and handling characteristics are suitable for using as an overlay when culturing gametes and embryos.

NidOil™ does not require washing before use, and it is neither too sticky nor too viscous, to facilitate pipetting.

Our stringent quality assurance control helps maintain our standard for low endotoxin levels and also ensures our products are free from microbiological contamination.

There have been several reports of paraffin oils becoming embryo-toxic after exposure to light on the laboratory bench. As a precaution against any possible light-induced changes, NidOil™ is packaged in amber, screw-top bottles.

A prospective randomized study to compare four different mineral oils used to culture human embryos in IVF/ICSOI therapy

Presented at ESHRE 2008 by Dr C. Sifer, Paris

Comparison between;

1. Mineral Oil (CryoBioSystem)

3. Liquid Paraffin (MediCult)

3. NidOil (Nidacon)

4. Ovoil (Vitrolife)

Group	1	2	3	4
No of cycles	129	126	126	119
GQE day 3	2.1	1.6	2.2	2.6
Impl. rate %	22.1	21.7	30.0	24.6
Clin. Pregn %	31	29	38	36

Recommendations before use

NidOil™ should be equilibrated in the same way as the culture medium before use to avoid differences in temperature and gaseous content between the components of the culture system.

New Quality Assurance test

Many questions has been raised lately to whether the oil that is used for coverage in embryo culture can actually damage the embryo.

All oil batches today from different manufactures are tested for sterility, endotoxins and a mouse embryo assay showing blastocyst development.This is apparently not enough since damage to cultures has been observed with an approved batch of oil.

One answer could be peroxidation of the oil which has been investigated in several publications and found to be harmful to fertilisation and embryo development when over a certain level.It has also been shown that peroxidase level in oil can increase over time, due to exposure to light or high temperature storage.

The peroxidase test in now included in our *Quality Assurance Certificate* which comes with every batch and we also test the raw material before we make the order.In this way we hope to be able to provide you with an oil for your cultures that is safe to use and still practical with the long shelf life and room temperature storage before opening.

If you have any questions regarding our tests, please let us know.

VitriBlast™ Kit ThermoBlast™Kit

Background

The formation of intracellular ice crystals is a major problem during the cooling and warming of cells. These ice crystals have detrimental effects on cell survival rates. Vitrification, which is the rapid freezing of cellular material, makes it possible to freeze cells while at the same time avoiding the formation of intracellular ice crystals. The use of the vitrification technique results in a very homogenous structure, an amorphous crystalline structure.

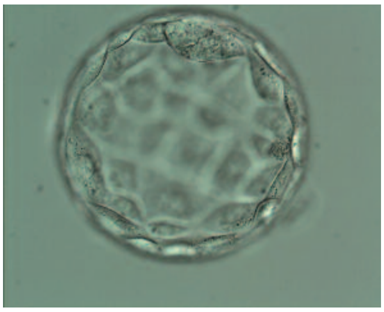
Recommendations

VitriBlast™ can be used with different types of vitrification-devices like the cryotop, the cryoloop or the high security straw (HSV). The method described below is with the cryoloop, but the same protocol can be used for all devices.

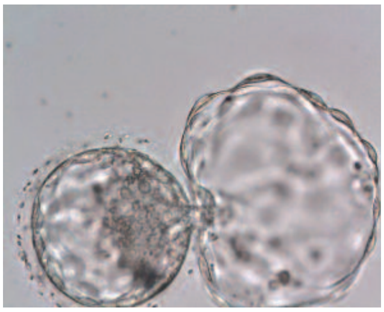
Work on a heated stage at all times when manipulating the blastocyst. Do not let the blastocyst remain exposed to the microscope light during incubation.

Reagents and Equipment

VitriBlast™ and ThermoBlast™ Kit
Sterile pipettes
Device for vitrification
CO_2 incubator
Stopwatch
Liquid nitrogen reservoir
Liquid nitrogen
Culture dishes (NUNC 4-well)
Heated stage
Inverted microscope



Vitrified and warmed blastocyst with excellent morphology.



Hatching blastocyst after vitrification and warming.

Vitrification procedure using the cryoloop

Additional equipment:
Cryocane for storage of cryo tubes
Crystalwand
Vial Clamp for holding the cryo tube
Cryoloop
(Hampton Research)

Note: If the additives are stored in the refrigerator, remove them in good time prior to use. DMSO turns solid below +18°C. The additives can be stored outside the refrigerator in the supplied packaging even after opening. If urgent the DMSO bottle can be warmed by holding the bottle in your hand.

1. Label the NUNC-dish with the patient ID and each well with corresponding solution number, i.e. 1, 2 and 3.
2. Pipette the vitrification media as described below. When adding the DMSO and Ethylene glycol (EG), which are included in the kit, to solutions 2 and 3, pipette the two up and down a few times to obtain optimal mixing of the media.

Well 1

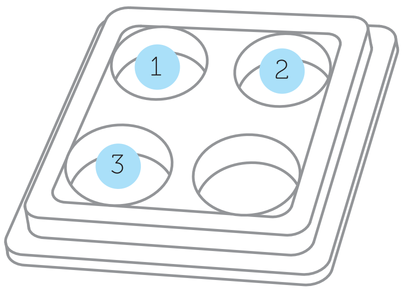
VitriBlast™1	1000 µL

Well 2

VitriBlast™2	850 µL
DMSO:	75 µL
EG:	75 µL

Well 3

VitriBlast™3	700 µL
DMSO:	150 µL
EG:	150 µL



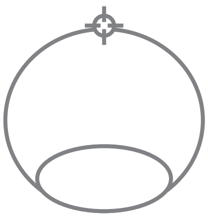
Vitrification procedure using the cryoloop

3. Incubate at 37°C in 5-6% CO_2 for 30 minutes, maximum for 1 hour. Longer time makes it difficult to create a film on the loop.

4. During the 30 minute incubation of the dish collapse the blastocyst. This can be done either by laser (Fertilase, red, 5, see pictures below) or by using an ICSI-pipette.

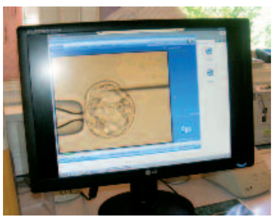
Laser

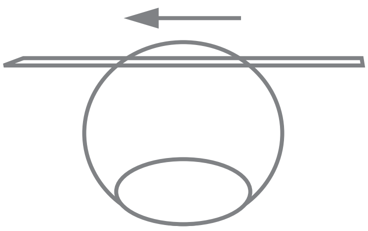
- If laser is used, shoot as far from inner cell mass (ICM) as possible. The laser beam shoots vertically, aim as illustrated below.
- Be sure that you create a hole through the zona and the trophectoderm.



ICSI-pipette

- If an ICSI-pipette or other sharp instrument is used, puncture right through the trophoblast cell layer into the blastocoele, and be sure to puncture as far as possible from the ICM.
- The pipette should be inserted at the one o´clock position and exit through the blastocyst at the11 o´clock position.





5. Remove the NUNC-dish from the incubator and place it on a heating stage (make sure the heat controller is set high enough to obtain 37°C in the media).

6. Place the punctured and collapsed blastocyst in solution 1. Start the stop watch.

7. After 1.5-2 minutes transfer the blastocyst by aspirating solution 2 into the pipette tip, collect the blastocyst from solution 1 and transfer it to solution 2.

8. Incubate on the heating stage for EXACTLY 2 minutes. Start the stopwatch and observe when 2 minutes is approaching. It is easier to start the stopwatch and let it run towards 2 minute. This removes the stress of the beeping noise when using a countdown timer. While incubating; proceed to step 9.

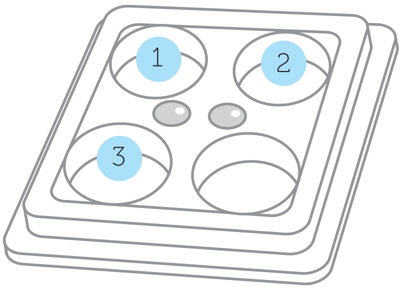
Do not let the blastocyst remain exposed to the microscope light during the incubation.

Vitrification procedure using the cryoloop

9. During the 2 minute incubation prepare 2 x 10 µL drops of solution 3 in the middle of the dish (see diagram below). The droplets evaporate quickly. It is better to prepare them as late as possible.

10. Attach the loop to the Crystalwand.

11. At the end of 2 minutes, transfer the blastocyst by aspirating solution 3 from the well into the pipette tip, collect the blastocyst from solution 2, and transfer it to solution 3 in the 10 µL droplet. The blastocyst must remain in solution 3 for **30–45 seconds**, including the time in the loop.



12. Coat the loop with solution 3 from the unused 10 µL droplet and place the blastocyst on the loop.

Note: Using drops reduces the risk of loosing the blastocyst. The blastocyst tends to float on the viscous solution 3. It is also important to incubate solution 3 under the same conditions as the other two solutions, hence the use of 1 mL.

13. Immerse in liquid nitrogen.

14. Attach the cryo tube to the Vial Clamp and immerse the tube in the liquid nitrogen allowing it to fill. Carefully insert the cryoloop into the tube and keep the cryoloop in liquid nitrogen during the entire procedure. Use the Crystalwand to close the tube.

15. Attach the tube to the Cryocane for storage in liquid nitrogen.

Warming procedure

1. Label the NUNC-dish with the patient ID and each respective well with each solution number, i.e. 4, 5, 6.

2. Pipette the warming media 4, 5 and 6 as described below.

Well 1

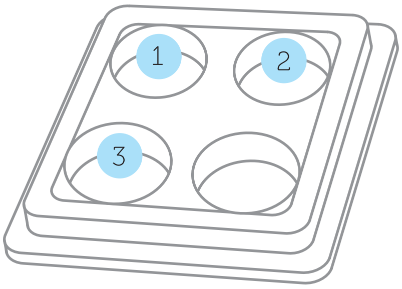
ThermoBlast™4: 1000 µL

Well 2

ThermoBlast™5: 1000 µL

Well 3

ThermoBlast™6: 1000 µL



3. Incubate at 37°C in 5-6% CO_2 for 30 minutes.

4. Carefully detach the cryoloop from the cryo tube, making sure not to touch the inside of the tube with the cryoloop (the blastocyst may be lost). Unscrewing the top and moving the cryoloop from the tube are the most risk-filled moments during this procedure.

5. Immerse only the loop in the surface of solution 4. Let the blastocyst fall off. Identify its presence in the well and incubate for 2 minutes on the heating stage. (2 minutes includes time for "locating" the blastocyst).

6. **Transfer** the blastocyst to solution 5. Let the blastocyst simply sink to the bottom, do not wash. Incubate for 3 minutes in solution 5.

7. **Transfer** the blastocyst to solution 6.

8. Incubate for 5 minutes.

9. Transfer the blastocyst to culture medium and allow the blastocyst time to reexpand. Wait 1 to 4 hours before final assessment. If the blastocyst has not re-expanded after 4 hours the chance of reexpansion is low.

Sperm VitalStain™

Background

Sperm vitality should be determined in semen samples with 50% or more immotile spermatozoa according to the WHO laboratory manual for the examination of human sperm.

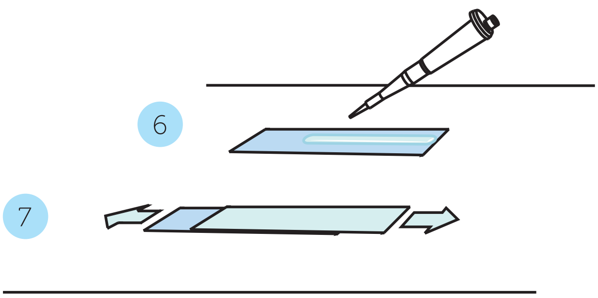
SpermVitalStain™ uses the eosin-nigrosine technique in a one-step method to establish the percentage of live spermatozoa. It is based on the principle that dead cells (i.e. those with damaged plasma membranes) will take up the eosin and stain red. Nigrosine provides the background to facilitate visualisation of the unstained (white) live cells.

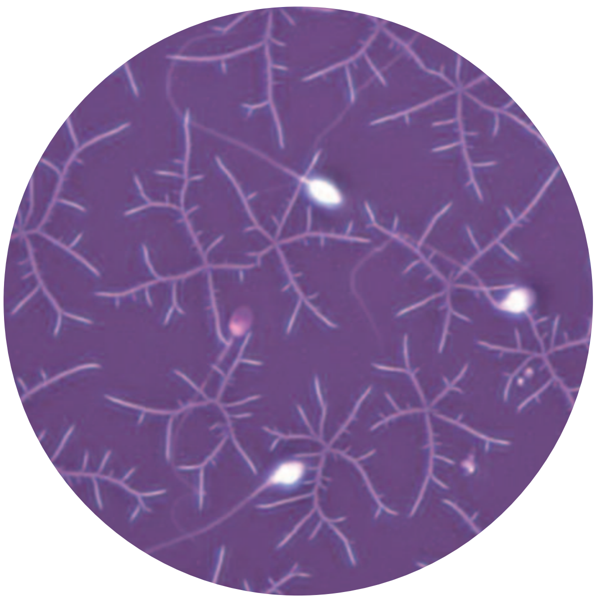
Reagents and Equipment

Light microscope (40 – 100 x magnification)
Microscope slides
Pipette
Test tube

Procedure

1. Shake the bottle of Sperm VitalStain™ before use.
2. Take an equal amount of Sperm VitalStain™ and the sperm sample (eg. 50 µL SVS + 50 µL sample). Use for example an eppendorf tube.
3. Mix well.
4. Leave for 30 seconds at room temperature.
5. Prepare a slide using your conventional method or use the method recommended by Nidacon.
6. Transfer a 20 µL drop onto a labelled microscope slide with a pipette, making a string/line of fluid in the middle of the slide.
7. Cover this slide with a second microscope slide and, when the drop is evenly spread between the two slides, pull them apart from each other horizontally. You then have two good slides.
8. Air dry the two slides and examine. If you want to store for later use, mount the slides with DPX or equivalent mountant, and a cover slide.
9. Examine using a bright field 40 x objective or a 100 x objective under oil immersion.
10. Count 200 sperm, the white (unstained) are classified as alive and the red or pink are classified as dead. Sperm coloured only at the neck region are classified as alive.





Tips

1 The 100x objective with immersion oil will give you a very clear picture of stained versus unstained sperm.

Sperm MorfoStain™

Background

The technique is based on the principle that sperm with different characteristics will stain so that one can differentiate between them. The numerical estimation of abnormal sperm in an ejaculate can aid in the judgement of whether, and which kind, of infertility treatment will be necessary.

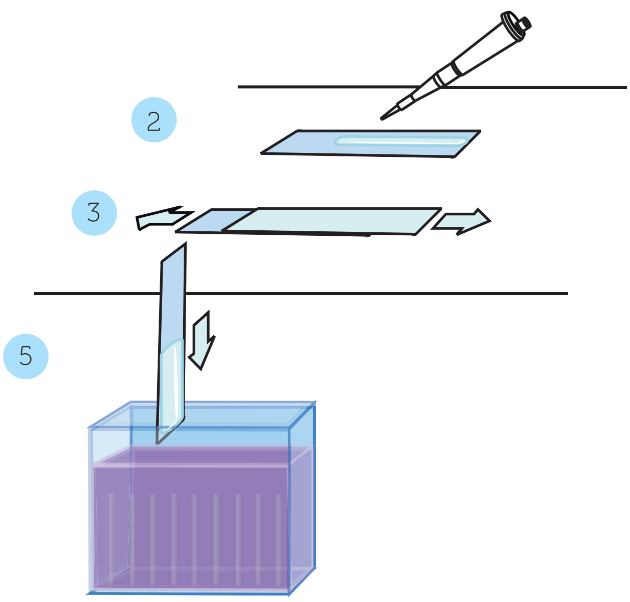
The sperm will be stained in a darker colour (blue) and the background will be lighter. Consequently, the shape, size and integrity of the sperm can easily be determined using 100x objective, oil immersion microscopy.

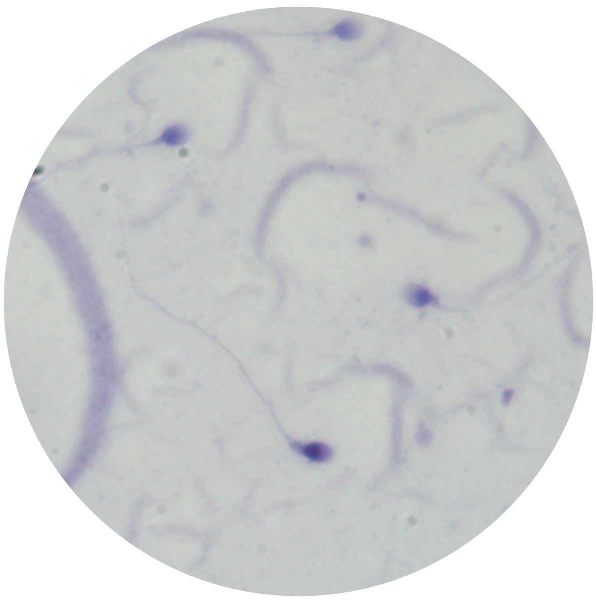
Reagents and Equipment

Coplin jar or similar
Microscope slides
Light microscope (40-100 x objectives)
Pipette

Procedure

1. Make a semen smear on a microscope slide using your conventional method or the method recommended by Nidacon.
2. Transfer a 20 µL drop onto a labelled microscope slide with a pipette, making a string /line of fluid in the middle of the slide.
3. Cover this slide with a second microscope slide and, when the drop is evenly spread between the two slides, pull them apart from each other horizontally. You then have two good slides.
4. Air dry the smears.
5. Dip the dry smears into the staining solution for 8 seconds.
6. Rinse in double distilled water, changing the water 3 times. Let slides air dry lying flat.
7. Mount the slides with coverslips and DPX, or equivalent mounting fluid, and let them dry completely before examination.
8. Examine using a bright-field 100 x objective under oil immersion.
9. Classify at least 200 sperm, classification according to the 2002 NAFA and ESHRE-SIGA manual on Basic Semen Analysis.





Tips

1 Use a pencil to mark your sample slides since the stain will remove permanent markers.

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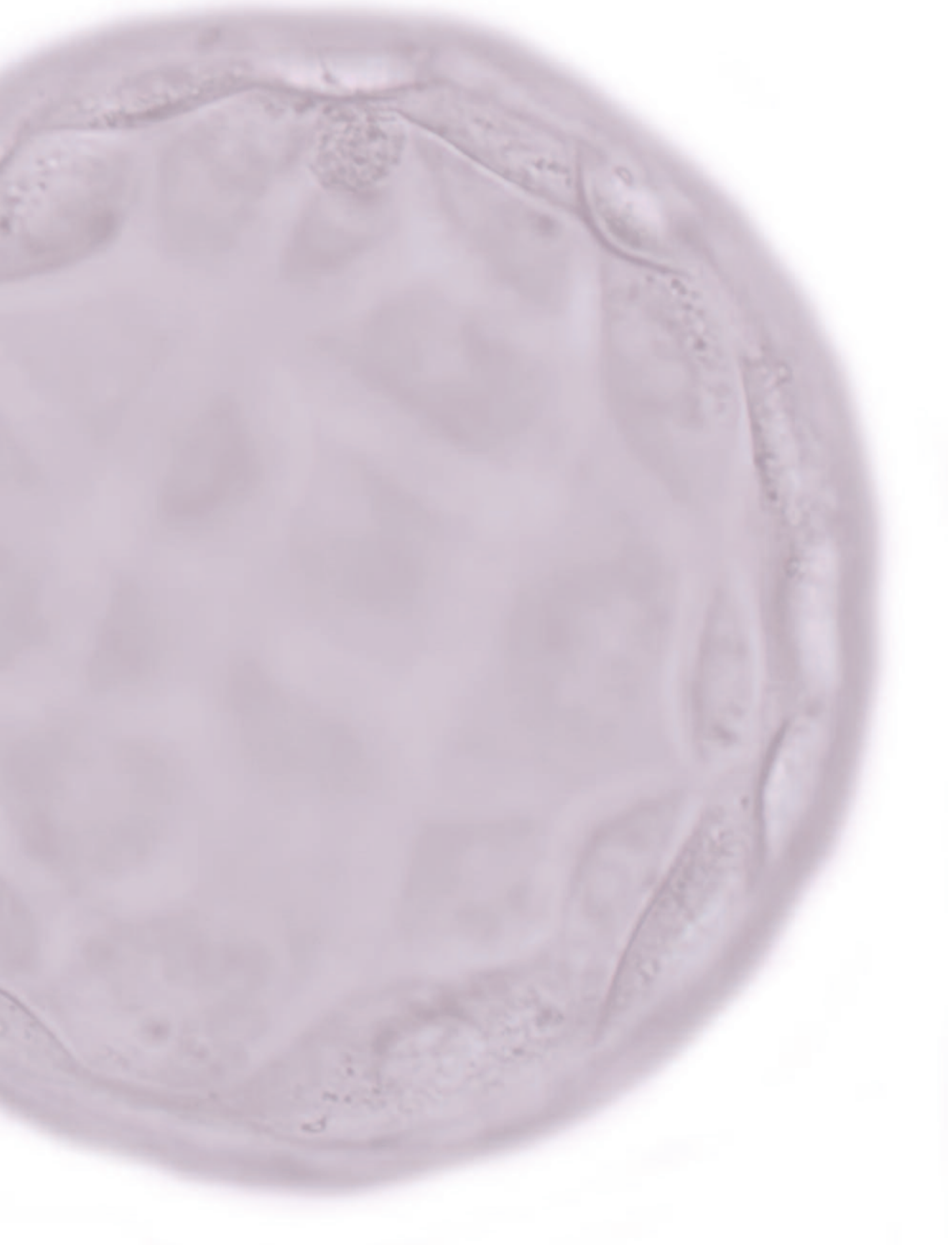

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